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*Published in:*
FEMS Microbiology Letters

*DOI:*
10.1111/j.1574-6968.1984.tb00324.x

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*Document Version*
Publisher's PDF, also known as Version of record

*Publication date:*
1984

Link to publication in University of Groningen/UMCG research database

*Citation for published version (APA):*
Elferink, M. G. L., Hellingwerf, K. J., Belkum, M. J. V., Poolman, B., & Konings, W. N. (1984). Direct interaction between linear electron transfer chains and solute transport systems in bacteria. *FEMS Microbiology Letters*, *21*(3), 293-298. https://doi.org/10.1111/j.1574-6968.1984.tb00324.x

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FEMS Microbiology Letters 21 (1984) 293–298
Published by Elsevier



# Direct interaction between linear electron transfer chains and solute transport systems in bacteria

(Solute transport, electron transport, proton motive force, regulation, *Rhodopseudomonas sphaeroides*, *Escherichia coli*, homeostasis)

Marieke G.L. Elferink, Klaas J. Hellingwerf, Marco J. van Belkum, Bert Poolman and Wil N. Konings

*Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands*

Received 21 October 1983
Revision received and accepted 18 November 1983

## 1. SUMMARY

In studies on alanine and lactose transport in *Rhodopseudomonas sphaeroides* we have demonstrated that the rate of solute uptake in this phototrophic bacterium is regulated by the rate of light-induced cyclic electron transfer.

In the present paper the interaction between linear electron transfer chains and solute transport systems was studied in *Rhodopseudomonas sphaeroides* and *Escherichia coli*.

The results demonstrate that the activities of alanine transport in *Rps. sphaeroides* and lactose and proline transport in *E. coli* are directly controlled by the electron transfer activity in the respiratory chain, under conditions that the proton-motive force remains constant.

## 2. INTRODUCTION

Solute transport in *Rps. sphaeroides* under anaerobic conditions in the light requires in addition to a proton-motive force, cyclic electron transfer [1]. Also transport systems like the lactose transport protein (M-protein) from *E. coli* introduced in the cytoplasmic membrane of the phototrophic bacterium via genetic manipulation are subject to this form of regulation [2]. As a result of this regulation of bioenergetic processes a pronounced homeostasis of the magnitude of the proton motive force is achieved in *Rps. sphaeroides*, allowing large variations in the rate of energy-consuming processes like solute uptake [1,2] or ATP synthesis (Elferink et al., unpublished results) with only minor variations in the magnitude of the proton-motive force.

The question arises whether this form of regulation of bioenergetic processes also occurs by linear electron transfer chains in *Rps. sphaeroides* and other bacteria or is confined solely to cyclic electron transfer chains. To answer this question we have measured the solute transport activity in *Rps. sphaeroides* and *E. coli* as a function of the activity of linear electron transfer chains.

## 3. MATERIALS AND METHODS

### *3.1. Pretreatment of* Rps. sphaeroides *cells*

*Rps. sphaeroides* strain 2.4.1. was grown anaerobically under high light intensity in the

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medium described by Sistrom [3] at 30°C. Cells were harvested at an absorbance at 660 nm of 3, washed twice at room temperature with 50 mM potassium phosphate pH 8.0, 5 mM $MgSO_4$, 50 $\mu$g/ml chloramphenicol and resuspended to a protein concentration of 13 mg/ml in the same buffer to which glutamate (1 g/l), malate (1.5 g/l), acetate (1.5 g/l) and succinate (1.5 g/l) were added. Nigericin (1 $\mu$M) was added to the cell suspension. Cells were stored at 4°C.

*3.2. Pretreatment of* E. coli *cells*

*E. coli* strain ML308-225 was grown aerobically at 37°C on minimal medium A [4] supplemented with 0.1% yeast extract and 0.5% glucose and harvested at an absorbance at 660 nm of 1.8. Cells were resuspended in 120 mM Tris–HCl pH 8.0 (0.1 g wet weight/ml) and incubated at 37°C for 2 min in 2 mM potassium-EDTA. The cell suspension was diluted 1 : 4 with 50 mM potassium phosphate pH 8.0, 5 mM $MgSO_4$ and 50 $\mu$g/ml chloramphenicol. The cells were washed twice and finally resuspended in this buffer to 30 mg protein/ml. Nigericin (1 $\mu$M) was added and the cells were stored at 4°C.

*3.3. Isolation of membrane vesicles*

*E. coli* strain ML308-225 was grown on minimal medium A [4] with 1% sodium succinate and 0.1% yeast extract. Right side out membrane vesicles were prepared as described by Kaback [5]. The vesicles were resuspended in 50 mM K-phosphate buffer pH 7, 5 mM $MgSO_4$ and 0.5 $\mu$M nigericine was added to the vesicles suspension.

*3.4. Simultaneous measurements of transport, membrane potential and rate of oxygen consumption*

A polyvinylchloride vessel of 3 ml was constructed in which both an ion-selective tetraphenylphosphonium ion ($TPP^+$) electrode and a Clark-type oxygen electrode were inserted. These electrodes allowed the simultaneous recording of the extracellular $TPP^+$ concentration and the rate of oxygen consumption. For uptake studies samples (50 $\mu$l) were withdrawn from the incubation mixture with a Hamilton syringe at different time intervals. The samples were directly diluted with 2 ml LiCl (0.1 M) and further handled as described [1]. In order to prevent inflow of oxygen the volume of the incubation mixture was decreased with 50 $\mu$l after every sample withdrawal via a screwcap cover of the vessel.

The initial rate of alanine uptake in anaerobically grown cells of *Rps. sphaeroides* and the initial rate of lactose and proline uptake in *E. coli* cells and membrane vesicles resp. were measured simultaneously with $\Delta\psi$ and respiration rate in this vessel. The initial rate of solute uptake was determined from the slope of the linear part of the uptake curve followed for several minutes. The first sample could be taken 20 s after the start of the experiment. The measurements in *Rps. sphaeroides* and *E. coli* cells were performed at 30°C and at pH 8.0 in the buffer described above. The cells were titrated with increasing amounts of KCN. The measurements in *E. coli* membrane vesicles were performed at 30°C and at pH 7 in the same buffer. The uptake rate was titrated with increasing amounts of oxamate.

*3.5. Analytical procedures*

Protein was determined according to the method of Lowry et al. [6].

*3.6. Calculations*

The magnitude of the membrane potential was calculated according to the Nernst equation. For all $\Delta\psi$ measurements a correction for $TPP^+$ binding was applied. For $\Delta\psi$ measurements in *Rps. sphaeroides* cells the correction was applied as described by Lolkema et al. [7,8]. For *E. coli* cells a correction for $TPP^+$ binding was applied based on the assumption that all binding occurs to the cytoplasmic membrane and is dependent on both the external and the internal probe concentration [8]. For *E. coli* membrane vesicles a correction for $TPP^+$ binding was applied by subtracting the amount of probe bound under de-energized conditions from the total amount of probe taken up under energized conditions.

*3.7. Materials*

D-Glucose-[1-$^{14}$C]lactose, L-[$^{14}$C]alanine and L-[$^{14}$C]proline were obtained from the Radiochemical Centre (Amersham, Buckinghamshire, UK). All other materials were of analytical grade.

## 4. RESULTS

In *Rps. sphaeroides* evidence for a direct interaction between cyclic electron transfer and solute transport was obtained by an analysis of flow-force relations of alanine uptake. The most informative way to present the data turned out to be a plot of the rate of solute uptake against the light intensity under conditions of constant proton-motive force [1,9]. If one assumes that the rate of turnover of cyclic electron transfer is a monotonic, saturating function of the light intensity then the conclusion is justified that the rate of solute (alanine) uptake — at constant magnitude of the proton-motive force — is modulated by the rate of cyclic electron transfer [1,9].

In linear electron transfer chains, with oxygen as terminal electron acceptor, the rate of electron transfer can be measured directly and consequently the rate of solute uptake can be plotted as a function of the rate of electron transfer (or: oxygen consumption) In the graphs presented the magnitude of $\Delta\psi$ is also indicated (or: the proton-motive force, since the $\Delta$pH was kept zero with nigericin (not shown) at the various rates of oxygen consumption.

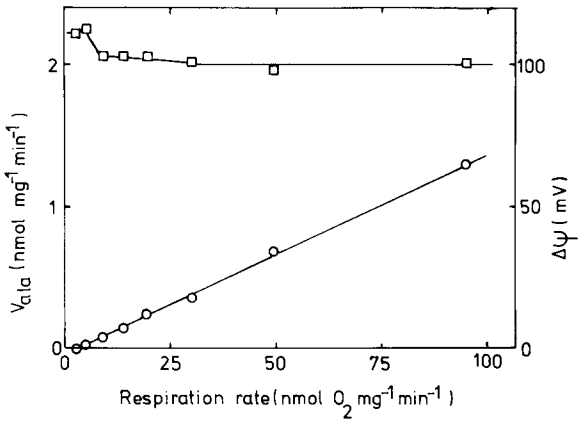


Fig. 1. The initial rate of alanine uptake and the magnitude of $\Delta\psi$ as a function of the respiration rate in anaerobically grown cells of *Rps. sphaeroides*. Anaerobically grown cells of *Rps. sphaeroides* were pretreated as described in MATERIALS AND METHODS. Alanine uptake, $\Delta\psi$ and respiration rate were measured simultaneously at 30°C and at increasing KCN concentrations. The incubation medium contained: 50 mM potassium phosphate pH 8, 5 mM $MgSO_4$, 50 $\mu$g/ml chloramphenicol, glutamate (1 g/l), malate (1.5 g/l), acetate (1.5 g/l), succinate (1.5 g/l), 50 $\mu$M L-alanine (10 Ci/mol), 4$\mu$M $TPP^+$ and the cells 1.3 mg proteins/ml. The KCN concentration was varied between 0 and 200 $\mu$M. ○, Initial rate of alanine uptake; □, membrane potential.

### *4.1. Regulation by linear transfer chains in* Rps. sphaeroides

*Rps. sphaeroides* cells, grown anaerobically in the light, were incubated in the dark in a buffer containing the carbon-sources from the Sistrom-medium. Under these conditions the rate of oxygen consumption was 95 nmol $O_2 \cdot$ mg protein$^{-1} \cdot$ min$^{-1}$. Respiration was progressively inhibited by the addition of increasing concentrations of potassium cyanide. The rate of alanine uptake decreased proportionally with the rate of respiration (Fig. 1). At about 4% of the maximal rate of respiration the alanine uptake was completely inhibited. The proton-motive force ($\Delta\psi$) remained essentially constant up to a cyanide concentration of 100 $\mu$M and subsequently increased slightly at higher cyanide concentrations. Similar results were obtained with aerobically grown *Rps. sphaeroides*. These cells were suspended in 50 mM K-phosphate buffer pH 8, 5 mM $MgSO_4$ and starved for 1 to 2 days before use by shaking in an erlenmeyer flask at 30°C. In the uptake experiments the cells were energized with ascorbic acid (1 mM) in the presence of TMPD (25 $\mu$M). Also in these cells, upon titration with cyanide the rate of uptake of alanine decreased roughly proportionally with the rate of electron flow while the proton-motive force remained essentially constant (not shown).

### *4.2. Regulation by a linear electron transfer chain in* E. coli

The rate of lactose uptake in *E. coli* cells was also measured at decreasing rates of respiration (Fig. 2). Also in this experiment the rate of respiration was inhibited with the oxidase inhibitor cyanide. Again the driving force for the uptake under these conditions remained approximately constant at cyanide concentrations between 0 and 5 mM. A remarkable decrease of the lactose uptake rate at decreasing respiration rates occurred. However, even at very low respiration rates a still significant uptake of lactose was observed. The

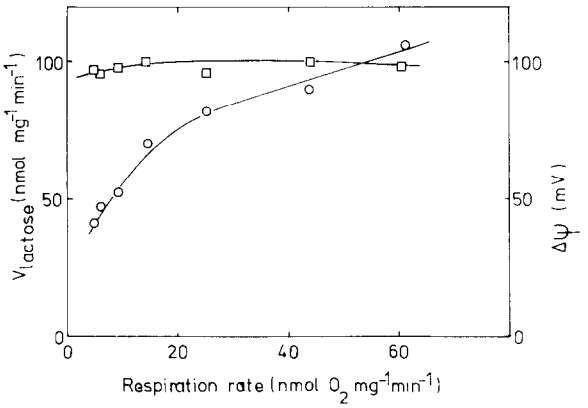


Fig. 2. The initial rate of lactose uptake and the magnitude of $\Delta\psi$ as a function of the respiration rate in cells of *E. coli*. Cells of *E. coli* were pretreated as described in MATERIALS AND METHODS. Lactose uptake, $\Delta\psi$ and respiration rate were measured simultaneously at increasing KCN concentrations. The incubation medium contained: 50 mM potassium phosphate pH 8, 5 mM $MgSO_4$, 50 $\mu$g/ml chloramphenicol, 200 $\mu$M lactose (4.2 Ci/mol), 4 $\mu$M $TPP^+$ and the cells 1.1 mg protein/ml. The KCN concentration was varied between 0 and 5 mM. ○, Initial rate of lactose uptake; □, membrane potential.

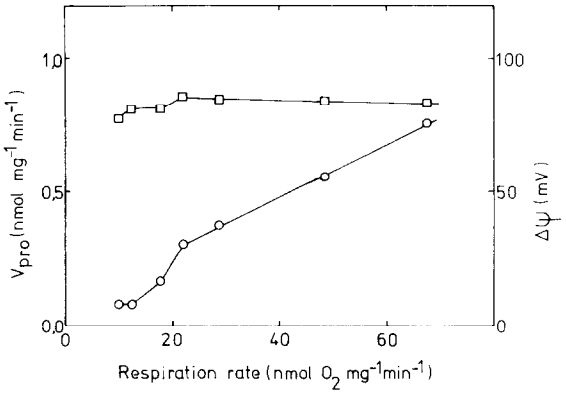


Fig. 3. The initial rate of proline uptake and the magnitude of $\Delta\psi$ as a function of the respiration rate in *E. coli* membrane vesicles. Membrane vesicles of *E. coli* were pretreated as described in MATERIALS AND METHODS. Proline uptake, $\Delta\psi$ and respiration rate were measured simultaneously. The incubation medium contained: 50 mM potassium phosphate pH 7, 5 mM $MgSO_4$, vesicles 2 mg protein/ml 4 $\mu$M $TPP^+$. After 3 min incubation with increasing amounts of oxamate (0 to 0.8 mM) 20 mM DL-lactate was added and after another minute the uptake experiment was started by adding 3.5 $\mu$M L-[$^{14}$C]proline (285 Ci/mol).

regulation of solute uptake by electron transfer through a linear electron transfer chain is also observed in membrane vesicles of *E. coli* (Fig. 3). In these membrane vesicles the oxidation rate of lactate was progressively inhibited with increasing concentrations (0 to 0.8 mM) of the lactate dehydrogenase inhibitor oxamate [10]. Again, the rate of uptake of the solute (proline in this case) correlated with the rate of linear electron transfer, whereas the driving force of the reaction under these conditions remained approximately constant.

To investigate the effect of turnover of the linear electron transfer chain on lactose uptake in more detail the uptake of lactose was measured at the transition from aerobic to anaerobic conditions (Fig. 4). In the presence of oxygen lactose was rapidly taken up and reached a steady-state level within 5 min. As soon as the oxygen was consumed in the incubation mixture, efflux of lactose occurred and a much lower steady-state level of lactose accumulation was obtained. The $\Delta\tilde{\mu}_{lactose}$ decreased from 156 mV to 112 mV. Upon the transition from aerobic to anaerobic state the $\Delta\psi$ increased slightly (see also below) from −82 mV in the presence of oxygen to −88 mV under anaerobic conditions.

If the uptake of lactose was started under

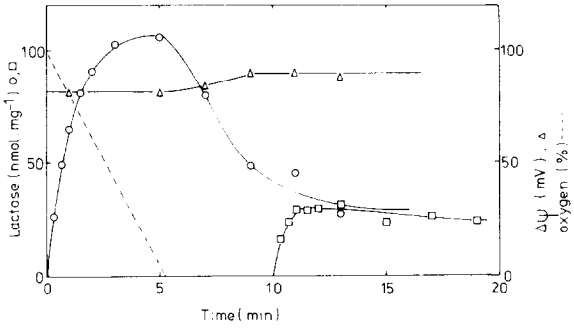


Fig. 4. Lactose uptake under aerobic and anaerobic conditions in cells of *E. coli*. Legend as in Fig. 2, except that no KCN was added to the incubation medium. The cells were present in a concentration of 0.8 mg protein/ml. ○–○–○, Lactose uptake during the transition from aerobic to anaerobic conditions; □–□–□, lactose uptake at anaerobic conditions; △–△–△, membrane potential; - - - - - -, percentage of the initial oxygen concentration.

anaerobic conditions the initial rate of lactose uptake was lowered from 79 nmol · min$^{-1}$ · mg$^{-1}$ in the presence of oxygen to 50 nmol · min$^{-1}$ · mg$^{-1}$. The $\Delta\psi$ remained constant at a value of $-88$ mV. The steady-state value for lactose uptake under anaerobic conditions was the same as before ($\Delta\tilde{\mu}_{lactose} = +112$ mV) (Fig. 4). Under the experimental conditions employed a small inflow of oxygen (maximally 2.5 nmol $O_2$ · min$^{-1}$ · min$^{-1}$) was unavoidable. If the uptake rates of lactose were measured under *strict* anaerobic conditions in a separate vessel with a constant flow of oxygen-free nitrogen over the surface of the incubation mixture, the uptake rate of lactose was 25 nmol · min$^{-1}$ · mg$^{-1}$ and the $\Delta\tilde{\mu}_{lactose}$ was $+80$ mV.

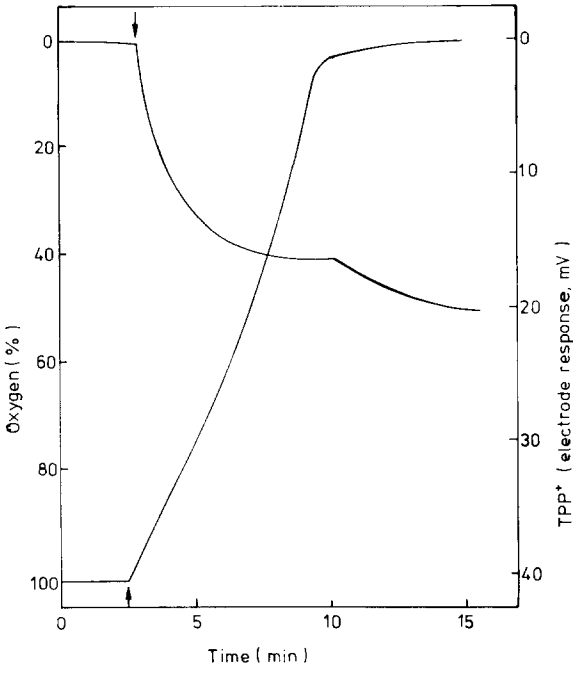


Fig. 5. Simultaneous recording of oxygen consumption and $TPP^+$ uptake in cells of *E. coli*. *E. coli* cells were pretreated as described in MATERIALS AND METHODS. The incubation medium contained 50 mM potassium phosphate pH 8, 5 mM $MgSO_4$, 50 μg/ml chloramphenicol. At the time indicated by the arrow cells were added to the incubation medium to a protein concentration of 1 mg/ml and the disappearance of oxygen and $TPP^+$ from the incubation mixture were directly recorded.

*4.3. Homeostatis of the proton-motive force in* E. coli cells

Fig. 5 shows a direct recording of the oxygen and tetraphenylphosphonium concentrations in a suspension of *E. coli* cells. In cells, free of inhibitors of respiration, the magnitude of the proton-motive force increased slightly upon the exhaustion of oxygen (when consequently the activity of solute transport systems decreases considerably). This increase in $\Delta\psi$ could be observed only when the cells were in 'optimal condition' i.e. grown on a rich medium, freshly harvested and not treated with dicyclohexylcarbodiimide. When the cells were incubated for 1 h at 37°C with 50 μM dicyclohexylcarbodiimide or when the cells were stored at 0°C for more than 2 days after harvesting, a decrease of the $\Delta\psi$ occurred upon transition from aerobic to anaerobic conditions. Omission of the yeast extract from the growth medium caused the same result.

## 5. DISCUSSION

In this paper we have presented evidence for a direct interaction between the electron transfer chains and solute transport in *Rps. sphaeroides* and *E. coli*. This regulation of the activity of solute transport systems was first observed in *Rps. sphaeroides* for the cyclic electron transfer system [1]. The results presented in this paper demonstrate clearly that this form of regulation is also exerted by linear electron transfer systems in *Rps. sphaeroides* and *E. coli*. Similar interactions between electron transfer systems and the membrane-bound ATPase complex were observed by Melandri and coworkers in *Rps. capsulata* [11], *Rps. sphaeroides* [12] and Jerusalem artichoke mitochondria [13]. These observations indicate that a direct interaction between electron transfer systems and energy transducing processes is a general property of energy transducing membranes.

The nature of this direct interaction is still unknown. In a previous publication [2] we have suggested that regulation by electron transfer might be exerted by an influence on the redox state of the transport proteins by the redox state of one or more electron transfer intermediates. Such a form

of regulation has been described previously for the protein kinases of chloroplasts [14]. In this interpretation the mechanism of energy transduction is chemiosmotic: the bulk phase electrochemical proton gradient is the driving force for solute transport but the activity of the transport system is regulated by the activity of the electron transfer system.

As an alternative to this: the direct interaction between electron transfer chains and solute carriers may be caused by specific proton conductance pathways on the surface of the membrane [15,16]. This interpretation would give the most straightforward explanation of the experiment described in Fig. 4. This experiment suggests that energy can be transduced via the direct interaction, because $\Delta\tilde{\mu}_{\text{lactose}}$ is considerably higher under aerobic conditions even though the proton-motive force under those conditions is slightly lower, compared to anaerobiosis. However, arguments against both interpretation for the molecular basis of the direct interaction can be brought forward [17].

As we have discussed for *Rps. sphaeroides*, the direct interaction between the rate of cyclic electron transfer and solute uptake (and other energy-consuming processes) causes a very pronounced form of homeostasis of the magnitude of the proton-motive force in intact cells. The results in *E. coli* suggest a similar type of regulation in this bacterium. Upon the exhaustion of oxygen the activity of solute transport systems — and possibly other energy-consuming processes too — decreased considerably. The proton-motive force on the other hand increased slightly.

## ACKNOWLEDGEMENTS

We thank Hans Westerhoff for clarifying discussions and Arend Abbing for expert technical assistance. This study was supported by the Netherlands Foundation for Chemical Research (SON) which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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